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# Challenges in Developing a Biochip for Intact Histamine Using Commercial Antibodies

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**Abstract:** This study describes the development and the challenges in the development of an on-chip immunoassay for histamine using commercially available antibodies. Histamine can be used as an indicator of food freshness and quality, but it is also a relevant marker in clinical diagnostics. Due to its low molecular weight, simple structure and thus low immunogenicity production of high specificity and affinity antibodies is difficult. From six commercial anti-histamine antibodies tested, only two bound the histamine free in the solution. A fluorescent on-chip immunoassay for histamine was established with a dynamic range of 8–111 µg/mL using polyclonal anti-histamine antibody H7403 from Sigma (Mendota Heights, MN, USA). The anti-histamine antibodies described and used in published literature are thoroughly reviewed and the quality of commercial antibodies and their traceability and quality issues are highlighted and extensively discussed.

**Keywords:** histamine; immunoassay; biochip; antibody quality; traceability; polyclonal

## 1. Introduction

Recent trends in food safety promote the use of biogenic amines as chemical indicator for the hygienic quality and freshness of foods. Biogenic amines can be produced during storage or product processing by bacterial enzymatic decarboxylation of free amino acids. Some of them, for example histamine, putrescine, cadaverine, spermine, spermidine and tyramine can be used as food quality indicator for bacterial spoilage and to monitor fermentation processes [1]. Histamine is especially important as it is toxic at high intakes and can be enhanced by the presence of other amines, such as putrescine and cadaverine. The limit value for fish and products thereof set by the FDA is 50 mg/kg [2]. Furthermore, biogenic amines have several important physiological functions in humans and animals and are also relevant markers for clinical diagnostics. Biogenic amines are traditionally measured using high pressure liquid chromatography (HPLC), which is an accurate but expensive and time-consuming method. A rapid, simple and low-cost test system that could be routinely used in industry is therefore urgently needed. Immunochemical methods provide a rapid analysis of a wide variety of target molecules, and immunoassays are routinely used in clinical diagnostics and several food and environmental applications. The main advantage of using antibodies is their sensitivity and selectivity; however, production of high specificity and affinity antibodies towards small molecules, haptens, such as histamine is challenging due to their small size and simple structure and thus low immunogenicity. Here, anti-histamine antibodies described and commercial antibodies used in published literature are reviewed to build the basis for the development of a rapid on-chip immunoassay for histamine.

### *1.1. Antibodies against Derived Histamine*

Anti-histamine antibodies are produced by either utilizing the histamine derivative or intact histamine to construct the immunogenic conjugate. When using derivatized histamine as immunogen the antibody recognizes not only the histamine residue but generally also the cross-linker and possibly part of the carrier protein. The histamine analyzed in the sample also needs to be derived prior the assay for specific recognition. In general, to obtain high affinity antibodies, haptens are often derived for antibody production. Several derivatization and conjugation methods are available for histamine–protein conjugation. Morel et al. [3] reviewed different ways of raising antibodies to histamine, and stated that high affinity monoclonal antibodies (mAb) could be raised only against derivatized histamine.

An acylating reagent, succinyl glycinamide *N*-hydroxysuccinimide ester, was used to derivatize histamine by Morel and Delaage [4]. The reagent synthesis required a laborious protocol. The mAbs raised against histamine-succinyl-glycyl-BSA conjugate exhibited high affinity with $K_d$ of $1.1 \times 10^{-10}$ M for acylated histamine, which was $5 \times 10^5$ greater than for native histamine. The usage of *p*-benzoquinone as the coupling agent between histamine and carrier proteins BSA or ovalbumin was presented by Guesdon et al. [5]. Benzoquinone enables the coupling of the histamine via its amino group to nucleophilic groups on proteins. The produced mAb showed high affinity towards the histamine-protein conjugate ($K_d$ of $4.6 \times 10^{-10}$ M) and histaminyl-benzoquinone ($K_d$ of $1.5 \times 10^{-8}$ M), which was more than $10^5$-fold higher than with free histamine. The limit of detection of the developed EIA for histamine was 4 nM. This monoclonal anti-histamine-benzoquinone antibody was used for determination of histamine in biological fluids [6] and food stuff [7]. Several histamine-protein conjugates were produced by Serrar et al. [8] by linking histamine to carrier protein using 1,4-benzoquinone or disuccinimidylsuberate. In addition, another coupling method applied required modification of both histamine and protein prior to the coupling: histamine was reacted with 2-iminithiolane (Traut's reagent), whereas the protein was reacted with SPDP (succinimidyl 3-(2-pyridyldithio)propionate) or sulfo-SMCC (sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate). Four of the produced mAbs bound intact histamine with $K_d$ of 15–57 mM, whereas the affinity enhancement of $10^5$ was achieved by derivatization of histamine.

### *1.2. Antibodies against Intact Histamine*

Even though obtaining sensitive antibodies against haptens often requires derivatization, antibodies recognizing intact histamine have been raised. The early studies exploited radioisotopic labels ($^3$H-histamine) [9–12], and mostly the raised antibodies were polyclonal (pAb) [9,11,12]. Haydik produced an antibody simply by absorbing histamine onto the proteins [3,9]. The resulting antibody recognized intact histamine, and its reported detection limit was 20 nM. Buckler et al. described in a patent [10] a synthesis of a *N*-alkyl immunogen of histamine, such as propionic acid derivative, and using carbodiimide as cross-linking chemistry for protein coupling. In the radioimmunoassay, the affinity towards intact histamine determined as $K_d$ was 500 nM. In another study, antiserum was raised against histamine conjugated to human serum albumin (HSA) using *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC) as a crosslinker [12]. Additionally, the conjugates were prepared using diisocyanate (toluene-2-isocyanate-4-isothiocyanate-4,4-diisocyanate). In the radioimmunoassay, the free intact histamine significantly inhibited antibody binding to histamine-HSA conjugate, resulting in $IC_{50}$-value (concentration which reduced antibody binding to histamine-HSA by 50%) of 3 mM. However, the affinity of the antibody for the conjugate used for immunization was significantly higher than for free histamine; when histamine was conjugated to HSA, $10^6$-fold lower amount compared to the free histamine was needed to result in equal inhibition. However, not all the attempts to produce specific antibodies against histamine or its derivatives have been successful and failed experiments have also been published highlighting the difficulty of the procedure [13]. Several studies describe the production

of anti-histamine antibodies and their usage in immunohistochemical studies [11,14,15]. In general, the host animal was immunized with histamine-protein conjugate prepared by using methylated protein carrier, glutaraldehyde as a linker or carbodiimide linking chemistries. The specificity of the antibodies was evaluated by abolishment of the immunostaining after pre-absorbing the antibody with histamine or other substances; however, high free histamine concentrations were used such as 1 mg/mL (9 mM) in [11].

Schneider et al. [16] were the first to develop a direct competitive enzyme immunoassay (EIA) utilizing a polyclonal histamine antibody that recognized intact histamine. The immunogen was prepared by conjugating histamine to carrier protein KLH with glutaraldehyde, and further used for immunizing a rabbit. A competitive assay was based on the immobilized anti-histamine antiserum and histamine-HRP conjugate as a tracer, which was prepared by reacting histamine towards HRP oxidized with sodium periodate. The assay was specific for intact histamine with a limit of detection of 230 ng/mL (2.1 μM) and $IC_{50}$-value of 520 ng/mL (4.7 μM), whereas cross-reactivity for histidine and other tested compounds related to histamine was less than 1%. These antibodies were later used in a competitive enzyme-linked immunosorbent assay (ELISA) for determination of histamine in cheese [17]. Furthermore, this antibody has been incorporated in an ELISA test kit commercialized by R-Biopharm GmbH (Darmstadt, Germany) [17]. Nevertheless, the repeatability of the method was discussed to be poor by Luo et al. [18], who despite several attempts were unable to produce an antibody recognizing intact histamine following the protocol described by Schneider and coworkers.

### *1.3. Commercial Anti-Histamine Antibodies and Their Use in Immunosensors and Tests*

Numerous anti-histamine antibodies are commercially available provided by several vendors. Both monoclonal and polyclonal antibodies are in the market and usually histamine conjugated to immunogenic carriers such as KLH or BSA has been used as an immunogen. The recommended applications are mainly immunohistochemistry (IHC) or immunocytochemistry (ICC), but also western blot, EIA and ELISA. Recently, several studies have pointed out the quality issues and traceability problem concerning commercial antibodies and the lack of quality in the antibody market has been widely recognized by the scientific community [19–28]. Often, the characterization and specifications provided by the manufacturers are somewhat incomplete and, furthermore, the authors often fail to identify the exact antibody used [28–30] for other researchers to be able to purchase the same product. Here, we reviewed published studies using commercial anti-histamine antibodies (excluding IHC and ICC applications).

A polyclonal anti-histamine antibody provided by Funakoshi Co. (Tokyo, Japan) was used in a histamine immunoassay employing SPR as detection platform [31]. Histamine was immobilized on gold surface via alkanethiol utilizing self-assembled monolayer, after which the assay was conducted in competitive manner using the purchased pAb. The affinity constants reported (with histamine $1.5 \times 10^7$ $M^{-1}$ and with immobilized histamine $7.2 \times 10^5$ $M^{-1}$) were relatively low, which was attributed to the polyclonality of the antibody. However, the limit of detection defined as 80–85% inhibition was 3 ng $mL^{-1}$ (27 nM). No derivatization of the histamine was needed. Adányi et al. [32] used the anti-histamine antibody provided by Sigma Aldrich in an optical waveguide lightmode spectroscopy (OWLS)-based immunosensor. The antibody was polyclonal, but it was mistakenly named as monoclonal in the publication (personal communication). The competitive assay, where histamine conjugated to BSA was immobilized on the functionalized sensor and competed for binding of the antibodies with intact histamine in the sample, was extremely sensitive with the limit of detection of 0.01 pg/mL (0.09 pM) for native histamine. Finally, histamine content in fermented vegetable juices was determined. Electrochemical flow immunoassay for histamine in blood samples was established using monoclonal anti-histamine antibody purchased from Biogenesis [33]. In the competitive immunoreaction histamine-IgG complex was separated from the histamine-BSA (coupled using 1,4-benzoquinone)-IgG complex, and the current change was measured using a multichanneled matrix column. Histamine in concentration range of ~200 to 2000 ng/mL was

detected. Another electrochemical immunosensor was based on the anti-histamine antibody from Sigma Aldrich immobilized onto graphene surface and the competition between the free histamine and HRP-histamine conjugate coupled with glutaraldehyde [34]. A linear current change was detected in histamine concentration range of 1 pg/mL–1 ng/mL with a detection limit of 0.5 pg/mL (4.5 pM). Anti-histamine antibodies have been incorporated into commercial kits based on ELISA method. However, the antibody raising methods are proprietary information and not provided. Many of the protocols require derivatization of the histamine in the sample prior to analysis, but derivatization reagents as well are company proprietary. Several derivatization methods utilize acylation of histamine. Commercial tests have been evaluated with real samples and compared their performance with methods such as the official AOAC method 977.13 for histamine or HPLC analysis. Properties of commercial test kits for histamine analysis are reviewed for instance in [35,36].

In this work, the aim was to develop an immunoassay for biogenic amine histamine. The availability of specific antibodies is often the main limiting factor when establishing a sensitive and specific immunoassay for small analytes such as histamine. Herein, we highlight the challenges in chip development based on commercial antibodies for intact histamine and extensively discuss the role of antibody quality and characterization and relating drawbacks.

## 2. Materials and Methods

### *2.1. Reagents*

Histamine dihydrochloride, spermine tetrahydrochloride, putrescine dihydrochloride, tyramine hydrochloride, histidine, Nω-acetylhistamine, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), horseradish peroxidase (HRP), bovine serum albumin (BSA), glucose oxidase (GOx), sodium borohydride, sodium periodate, sodium hydroxide, glutaraldehyde, sodium acetate, and CF™ 647 labeled anti-rabbit IgG produced in goat (SAB4600185, Lot 14C0331) were purchased from Sigma Aldrich (St. Louis, MO, USA). Keyhole limpet hemocyanin (KLH) was obtained from Thermo Fisher Scientific (Waltham, MA, USA) and BSA-histamine conjugate from Gentaur Molecular Products (Brussels, Belgium). PD-10 Desalting Columns were from GE Healthcare Life Sciences (Marlborough, MA, USA). Enrofloxacin hydrochloride was purchased from Toku-E (Bellingham, WA, USA). As slide substrate proprietary ARChipEpoxy slides (EP 02799374) were used. Anti-histamine antibodies used in the study are listed in the Table 1.

The following buffers were used: Assay buffer (0.1 M Tris, 0.1 M NaCl, 0.01 M $CaCl_2$, 0.1% Tween-20; pH 7.4), 10 mM phosphate buffered saline (PBS, pH 7.4, Invitrogen, Carlsbad, CA, USA), Nexterion I (Schott, Mainz, Germany), and MES (0.1 M MES, 0.9% sodium chloride, pH 4.7, Thermo Fisher Scientific, Waltham, MA, USA).

### *2.2. Microarray (Biochip) Fabrication*

Proprietary ARChip Epoxy glass slides (25 mm × 75 mm) were used as a chip platform to generate an ordered grid of protein-histamine conjugates. The conjugates were deposited in triplicates by contact spotting and the spotted slides were mounted into hybridization cassettes to create 12 array fields (7 mm × 7 mm each). Each array representing one experiment was incubated with histamine spiked sample to perform the assays as schematically presented in Figure 1. After the assay was completed the chip was read-out using a fluorescence scanner. This methodology is well established in our lab for medical diagnostics [37,38] and food analysis [39] and is described in more detail in Sections 2.2.1–2.2.4.

**Table 1.** Commercially available anti-histamine antibodies used in this study and their specificity as described by the suppliers.

| Code | Retailer | Catalog Number | Lot/Batch | Host | Clonality | Immunogen | Purity | Cross-Reactivity/Specificity | Application | RRID [40] |
|---|---|---|---|---|---|---|---|---|---|---|
| Ab1 | Sigma Aldrich | H7403 | 081M4794, 51K4855 | rabbit | pAb | histamine conjugated to succinylated KLH | affinity isolated antibody | stains histamine-containing cells in fixed sections of rat endocrine cells and mast cells | dot blot, IHC | AB_260077 |
| Ab2 | ImmunoStar | 22939 | 1006001 | rabbit | pAb | synthetic histamine coupled to succinylated KLH with carbodiimide linker | whole serum | staining blocked by pre-absorption of the antiserum with histamine conjugate no cross-reactivity with L-histidine | IHC | AB_572245 |
| Ab3 | Thermo Fischer Scientific | PA1-85547 | OD1701064 | rabbit | pAb | synthetic peptide corresponding to part of the native molecule conjugated to BSA | Protein G purified | detects histamine in many different sample types | ELISA | AB_931244 |
| Ab4 | Biozol Diagnostica Vertried/AbD Serotec | 4970-0010 | 0812 | rabbit | pAb | histamine conjugated to cationised BSA using a 1,4-benzoquinone | affinity chromatography on protein G | specific for histamine | ELISA | AB_619446 |
| Ab5 | Bertin Pharma | A03890 | 0116 | mouse | mAb | histamine-NH-CO-(NH2)6-*S*-maleinido-BSA | | histamine 100% 3-methyl histamine, 1-methyl histamine, histidine, serotonin <0.04% | EIA | |
| Ab6 | Merck Millipore | MAB5408 | 2522068 | mouse | mAb | histamine-hexamethylene diisocyanate (HD)-BSA | purified | histamine-HD-BSA 1 1-methyl-histamine-HD-BSA 1 histamine-GA-BSA, histidine-HD-BSA 1/>50 000 | IHC, ICC | AB_177651 |

#### 2.2.1. Preparation of Protein-Histamine Conjugates

Histamine was coupled to the carrier proteins for immobilization on the chip. For carbodiimide-coupled conjugates 3.3 mg histamine dihydrochloride (corresponds to 2 mg histamine) was mixed with 1 mg of protein (HRP, BSA, GOx, and KLH) in the presence of 10 mg of EDC in 700 µL of 0.1 M MES (pH 4.7) for 2 h at room temperature (RT). The conjugates were named as protein-EDC-his, respectively.

HRP(kit)-his conjugate was prepared using EZ-Link™ Plus Activated Peroxidase -kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Briefly, 1.7 mg of histamine dihydrochloride was dissolved in 1 mL of PBS, and added into 1 mg of lyophilized HRP reconstituted in 100 µL of ultrapure water. After addition of 10 µL of sodium cyanoborohydride (5 M), the solution was incubated for 1 h in slow shaking. Finally, quenching buffer (20 µL of 3 M ethanolamine) was added and the solution was incubated additional 15 min.

GOx was oxidized with sodium periodate based on the protocol by Strasser et al. [41]. First, 2 mg of GOx was dissolved in 1.75 mL of 0.1 M sodium acetate (pH 5.4) and added to a vial containing 5 mg of $NaIO_4$. After incubation for 30 min at RT the reaction was stopped by purification with PD-10 Desalting Columns. Histamine dihydrochloride (8.3 mg) was added to the solution of activated protein and the pH was adjusted to 9.5 with 1 M NaOH. The reaction was incubated for 2 h, after which 100 µL of $NaBH_4$ (12 mg/mL in MQ) was added. The mixture was incubated for 1.5 h at 4 °C and purified as above.

HRP(oxidized)-his conjugate was prepared with the modified protocol from Schneider et al. [16]. HRP (27 mg in 2 mL of water) was activated by incubation with 0.4 mL $NaIO_4$ (21.4 mg/mL) for 30 min. The protein was purified with PD-10 column against 10 mM acetate buffer (pH 4.4). Histamine dihydrochloride (3.3 mg in 200 µL water) was added and subsequently the pH was adjusted to 9.5. After 2 h of incubation in RT, 0.1 mL of $NaBH_4$ (4 mg/mL in water) was added and incubated for 1 h in RT. The conjugate was purified with PD-10 purification column against PBS.

HRP- and BSA-GA-his conjugates were prepared with the modified protocol from Fujiwara et al. [42]. Histamine dihydrochloride (12 mg) was dissolved in 0.2 M sodium acetate (pH 5.4), and 1 mL of 30 mM glutaraldehyde (GA) was added. After incubation of 30 min HRP or BSA (10 mg) was added in 1 mL of acetate buffer. After incubation of 15 min at RT, 5 mg of $NaBH_4$ was added to saturate the double bonds. The conjugate was purified with PD-10 purification column against PBS.

#### 2.2.2. Spotting of the Probes

Conjugates and pure proteins were spotted in Nexterion I buffer on epoxy slides using protein concentration of 0.5 mg/mL. The spotting was carried out with Nanoprint Protein Microarrayer (Arrayit, Sunnyvale, CA, USA) using SMP3 pin (Arrayit). After spotting, the slides were kept at 4 °C for at least 72 h to facilitate full immobilization of the probes.

#### 2.2.3. Histamine Immunoassays

The reactive groups on the surface of the spotted slides were blocked with 1× PBS containing 0.1% Tween-20 in continuous stirring for 30 min. Slides were washed twice with PBS after which they were dried with compressed air. A hybridization cassette (FAST frame multiwell chambers from Whatman Ltd., Maidstone, UK) was used to form separated wells on the slide. Washing steps were done with PBS supplemented with 0.1% Tween-20 and finally in PBS.

In the simulated assay, anti-histamine antibodies were added in duplicates from 42 to 1500 ng/mL or 2000 ng/mL in assay buffer. Reactions were incubated in slow shaking for 2 h protected from light. After washing 50 µL of fluorescently labeled anti-rabbit IgG was added and incubated for 45 min in slow shaking. Finally, the wells were washed and the slides dried with compressed air. Binding inhibition immunoassay was carried out similarly except histamine in the concentration range of 3.75–600 µg/mL was pre-incubated with anti-histamine antibody for 30 min in slow shaking before

applying 50 µL of the sample into the assay wells. Antibody concentration for the binding inhibition assay was selected based on the simulated assay (900 ng/mL for Ab1 and 1/250 dilution for Ab2).

The cross-reactivity of the Ab1 in the binding inhibition assay was studied by pre-incubating other analytes instead of histamine. Following analytes were tested: tyramine, spermine, putrescine, enrofloxacin, histidine and histamine derivative Nω-acetylhistamine in the same concentration range than histamine.

#### 2.2.4. Detection

Fluorescence detection was conducted using Tecan LS Microarray scanner with excitation at 633 nm. The average signal of three replicate spots was analyzed with GenePix software (Molecular Devices, Sunnyvale, CA, USA) and further processed in Microsoft Excel and OriginPro (Origin-Lab, Northampton, MA, USA). The sensitivity of the assay was reported as the histamine concentration in which the antibody binding to immobilized histamine-protein conjugate was inhibited by 50%. The dynamic range was calculated as histamine concentrations providing normalized signals in the range of 20–80%.

## 3. Results

### *3.1. Assay Format*

In the binding inhibition assay, the analyte (histamine) free in solution competes with the immobilized antigen for the antibody binding sites. The bound anti-histamine antibody is then detected with a labelled secondary species specific antibody and the fluorescence readout is measured. The schematic assay construction is depicted in Figure 1. The binding of the antibodies towards the immobilized antigens (probes) was studied with a simulated assay, where increasing amounts of antibody was added without the analyte.

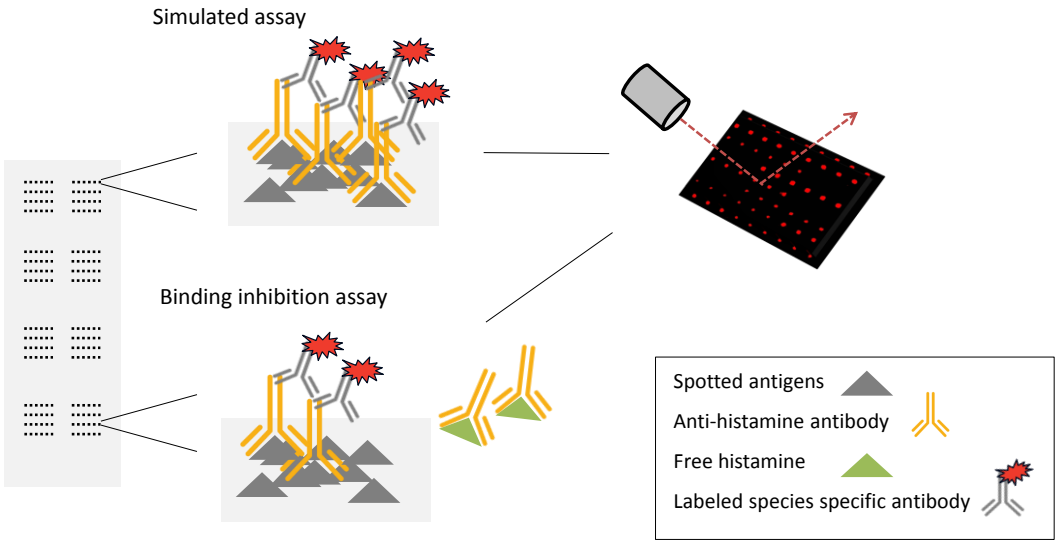


**Figure 1.** Schematic illustration of the simulated and binding inhibition assay for histamine. The antigens spotted on the microarray are bound by the anti-histamine antibody, the binding of which is inhibited by the free analyte in the solution. The bound antibodies are detected with a labeled species specific antibody, and the read-out is carried out with a fluorescence scanner.

### *3.2. Choice of Antibodies*

The performance of an immunoassay depends on the selectivity and affinity of the antibody towards the analyte, thus the selection of the antibody is a crucial step for the assay development. First step in the assay development was to find probes binding the antibodies. Therefore, a set of six

commercial antibodies, a representative mix of antibodies of different immunogens and clonality was tested in combination with several histamine-conjugates. The tested antibodies are listed in Table 1, and the histamine-conjugates including a short description of their preparation are compiled in Table 2. Different carrier proteins and different coupling chemistries were used to account for the different types of immunogens and the antibody specificity related with it. The chip containing the different conjugates deposited in 150 µm spots was incubated with increasing amount of antibodies (in a so called simulated assay) and the binding was detected utilizing a fluorescently labeled corresponding species specific antibody.

The signal of the probes indicated in Table 2 increased with increasing concentrations of antibodies added. Comparison of the antibodies was carried out using BSA-EDC-his as a probe because it was bound by four antibodies out of the six tested ones (Figure S1). This was not achieved for any other probe. Ab1 revealed the highest analytical sensitivity, while the dynamic range was similar for all tested antibodies. In general, the standard deviations increased with increasing concentrations of the antibodies. The average coefficient of variation (CV) was the lowest for Ab2 (4%), and highest for Ab4 (17%). The monoclonal anti-histamine antibodies did not bind to any of the spotted conjugates, whereas all tested polyclonal antibodies bound some of the conjugates (Table 2). This can be explained by the differences in the antibody production; polyclonal antibodies represent a pool of antibodies against an immunogen with a generally higher probability to bind an epitope under different conditions, while a monoclonal antibody is more likely to function in only one set of conditions. Polyclonal antibodies Ab1 and Ab2 bound most of the spotted conjugates.

**Table 2.** Binding of the anti-histamine antibodies from different manufacturers to probes immobilized on the epoxy slide surface. The sign + indicates that the antibody bound the probe, while − indicates that no antibody was bound. Probes tested with all the antibodies are highlighted in gray. Dynamic range and $IC_{50}$ in binding inhibition assay is shown for Ab1. The dynamic range was calculated as histamine concentrations providing normalized signals in the range of 20–80%.

| | Ab2 | Ab3 | Ab4 | Ab5 | Ab6 | Ab1 | Dynamic Range (µg/mL) | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|---|---|
| **carbodiimide coupling chemistry** | | | | | | | | |
| BSA-EDC-his | + | + | + | − | - | + | 13–106 | 37 |
| HRP-EDC-his | + | − | − | − | − | + | 8–111 | 28 |
| KLH-EDC-his | + | − | − | − | − | + | 11–103 | 48 |
| GOx-EDC-his | + | n/a | n/a | − | n/a | + | 16–98 | 37 |
| pure proteins | | | | | | | | |
| BSA pure | + | − | − | n/a | n/a | − | | |
| HRP pure | + | n/a | n/a | n/a | n/a | − | | |
| KLH pure | + | n/a | n/a | n/a | n/a | − | | |
| GOx pure | − | n/a | n/a | n/a | n/a | − | | |
| **aldehyde-based coupling** | | | | | | | | |
| HRP(kit)-histamine | − | − | − | − | − | + | 4–89 | 20 |
| GOx(oxidized)-his (acc. Strasser) | − | n/a | n/a | n/a | n/a | + | | |
| HRP(oxidized)-his (acc. Schneider) | n/a | n/a | n/a | n/a | n/a | − | | |
| **glutaraldehyde as a cross-linker** | | | | | | | | |
| BSA-GA-his | − | n/a | n/a | n/a | n/a | + | 28–191 | 70 |
| HRP-GA-his | n/a | n/a | n/a | n/a | n/a | + | 37–336 | 85 |
| commercial BSA-his conjugate | − | − | + | − | − | + | 4–38 | 14 |

The antibodies were further subjected to the binding inhibition assay, where a constant amount of antibody was pre-incubated with histamine (up to 600 µg/mL) and further applied onto the immobilized probes. Binding of free histamine by the antibody was only observed for Ab1 and Ab2. As presented in Figure 2, binding of Ab1 to the probes was inhibited by histamine more efficiently than the binding of Ab2. The signal-to-background (sg/bg, defined as ratio of the signal from zero analyte concentration to the signal of inhibited binding obtained at the highest analyte concentration) varied for Ab1 from 3.5 for KLH-EDC-his to 5.9 for HRP-EDC-his. For Ab2, the sg/bg was low, ranging from 1.2 for KLH-EDC-his to 2.6 for BSA-EDC-his.

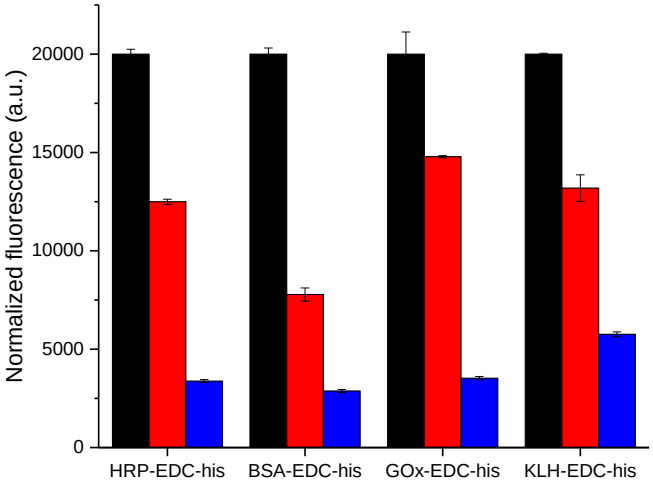


**Figure 2.** Normalized fluorescence signals of the binding inhibition assay. The black column represents the normalized signal of the zero analyte concentration. The inhibited antibody binding using 600 μg/mL of histamine as an analyte is shown in red for Ab2 and in blue for Ab1.

Ab3 and Ab4 did not recognize free histamine. The paratopes of the antibody were not blocked by histamine and the binding to the probes immobilized on the chip was not inhibited in the presence of high amount of histamine (600 μg/mL); instead, these antibodies bound the immobilized probes regardless of the amount of the added histamine. On the contrary, Ab5 and Ab6 were not bound to any of the probes included on the chip.

### *3.3. Specificity of the Antibody Binding in Simulated Assay*

In Section 3.2, we demonstrated that Ab1 and Ab2 were the only antibodies that bound to both the selected probes and to free histamine. To evaluate whether this binding was specific, we first studied the interaction of Ab1 and Ab2 with the pure carrier proteins (HRP, BSA, KLH, and GOx) and histamine-protein conjugates immobilized on the ARChip Epoxy platform. Conjugates were prepared with aldehyde-based coupling, or using carbodiimide and glutaraldehyde as cross-linkers.

Both Ab1 and Ab2 were produced using histamine coupled to succinylated KLH with carbodiimide linker as immunogen. We therefore evaluated the response to immobilized conjugates prepared of proteins also different from KLH (BSA-EDC-his, HRP-EDC-his, and GOx-EDC-his) and by using different coupling chemistries (EDC, aldehyde, and glutaraldehyde). Ab1 bound to most of the tested conjugates in dose-response manner (Table 2). All protein-EDC-his probes were bound similarly (Table 2), thus HRP-EDC-his was selected for further analysis. Furthermore, the commercial BSA-his and HRP(kit)-his resulted in similar dose-response compared to protein-EDC-his probes (Figure 3a). The average CV% of HRP-EDC-his was 8%, whereas with commercial BSA-his and HRP(kit)-his CV% were 15–17%. Similar to $IC_{50}$-value, we estimated the binding affinity by comparing the concentration at which normalized signal of 10,000 a.u. was achieved (half of the signal obtained with highest Ab concentration). The highest affinity was obtained against HRP-EDC-his (228 ng/mL), as for commercial BSA-his and HRP(kit)-his the values were 274 and 276 ng/mL, respectively. The Ab1 exhibited less affinity towards the probes GOx(oxidized)-his (725 ng/mL), as well as towards glutaraldehyde-coupled conjugates HRP-GA-his (550 ng/mL) and BSA-GA-his (469 ng/mL) (Figure 3b).

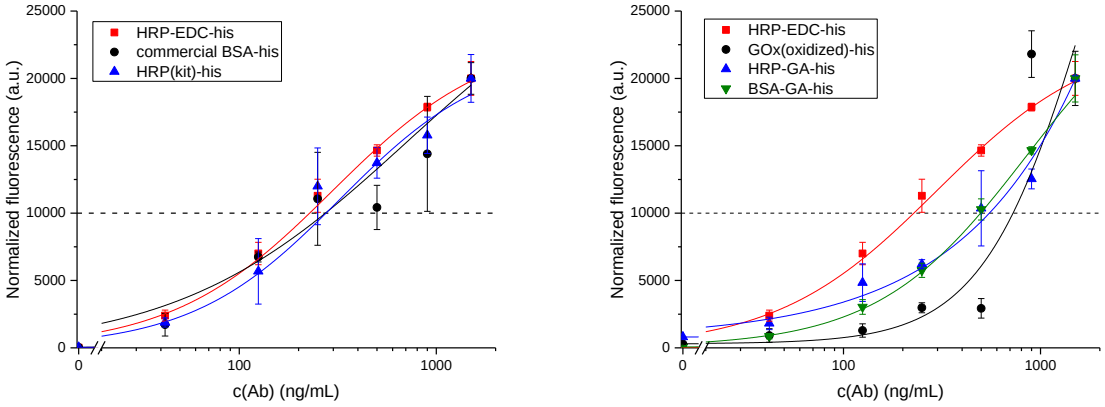


**Figure 3.** Normalized fluorescence response in simulated assay upon addition of increasing amounts of Ab1 onto the selected probes: (**a**) HRP-EDC-his, commercial BSA-his and HRP(kit)-his; and (**b**) HRP-EDC-his, GOx(oxidized)-his, HRP-GA-his, and BSA-GA-his. The signal level of 10,000 is indicated with a dashed line.

However, Ab1 did not bind to the respective free proteins while Ab2 bound them to high extent. Ab1 bound the respective protein-EDC-his conjugate more efficiently than the pure carrier protein: for KLH, 4%; for GOx, 7%; for HRP, 9%; and for BSA, 20% binding compared to corresponding protein-EDC-his conjugate were observed (Figure S2). The Ab2 bound the pure proteins up to 53–78% (in case of BSA, GOx or KLH) and 29% in the case of HRP, respectively.

### *3.4. Binding Inhibition Assay Using Ab1*

A binding inhibition assay for histamine was established using Ab1. Histamine-conjugates prepared with carbodiimide chemistry (BSA/HRP/KLH/GOx-EDC-his), HRP (kit)-his, GOx(oxidized)-his, BSA/HRP-GA-conjugates and commercial BSA-his conjugate were used as probes spotted onto ARChip Epoxy. The antibody concentration was titrated in the simulated assay (Figure 3) and based on that two concentrations were selected for the binding inhibition assay: 900 ng/mL and 250 ng/mL. With the lower antibody concentration, the signals were naturally lower, but could be easily adjusted by changing the measurement parameters of the fluorescence scanner (increasing the voltage of the photomultiplier tube (PMT)). Despite different signal levels the change in antibody concentration had only a little effect on the assay performance in terms of the $IC_{50}$ or dynamic range. For instance, in the case of HRP(kit)-his with 250 ng/mL of Ab1 the dynamic range was 35 µg/mL narrower and the $IC_{50}$ was lower by 13 µg/mL. The relatively small differences might be because the "dynamic range" in the simulated assay was relatively narrow. Likewise, the choice of the carrier protein in EDC-conjugates did not have any effect on the binding. The normalized response to these probes was the same regardless the carrier protein, thus the HRP-EDC-his is shown. The standard curves are shown in Figure 4. The dynamic range was similar with all the probes prepared with carbodiimide chemistry. With commercial BSA-his conjugate the dynamic range was narrower (4–38 µg/mL), as well as with HRP(kit)-his (4–89 µg/mL) (Table 2). Using $IC_{50}$ as an index for assay sensitivity, the most sensitive detection was achieved with commercial BSA-his (14 µg/mL), however the sensitivity with HRP(kit)-his (20 µg/mL) and HRP-EDC-his (28 µg/mL) were in similar level. When using glutaraldehyde-conjugates sensitivity was lost: with BSA-GA-his the $IC_{50}$ was 70 µg/mL and HRP-GA-his 85 µg/mL, respectively. Nonetheless, the dynamic range was wider using glutaraldehyde-coupled conjugates compared to protein-EDC-his probes; from 37 µg/mL up to 336 µg/mL with HRP-GA-his. Protein-EDC-his probes showed the highest signals in zero analyte concentration. The signals of the commercial BSA-his conjugate varied significantly compared to the other probes, up to 40% between the independent experiments, nevertheless the maximal

signal reached levels of 93% of the HRP-EDC-his signal. The other probes exhibited lower signals. Compared to the signal of HRP-EDC-his probe their signal levels were, for each probe: HRP(kit)-his, 72%; KLH-succ-EDC-his, 21%' BSA-GA-his, 19%' GOx(oxidized)-his, 5%; and HRP-GA-his, 4%. Even though the absolute signal levels varied within the probes, the differences could be simply compensated by adjusting the measurement settings, i.e., the voltage of the photomultiplier tube. However, the most challenging aspect of the conjugates was their stability and their performance in the assays regarding the repeatability. GOx(oxidized)-his broke down within two weeks, and the glutaraldehyde conjugation chemistry resulted in high variation within the assay in addition to the lower sensitivity compared to the other probes. Furthermore, the commercial BSA-his and HRP(kit) required freshly spotted slides to be used. In terms of stability the protein-EDC-his conjugates showed the highest stability and the probes spotted onto slides were reproducibly usable even after five months of storage.

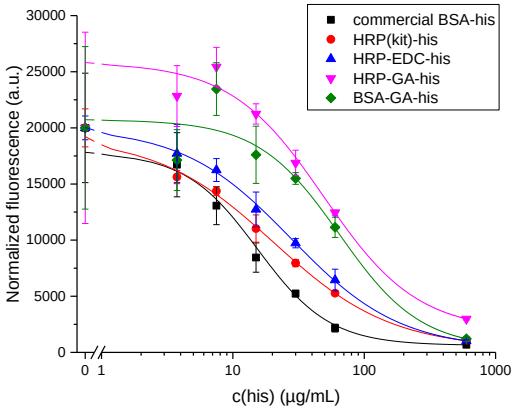


**Figure 4.** Normalized signals of the binding inhibition assay using Ab1 on selected probes: commercial BSA-his, HRP(kit)-his, HRP-EDC-his, HRP-GA-his, and BSA-GA-his.

Moreover, cross-reactivity with Ab1 was tested using tyramine, spermine, putrescine, enrofloxacin, histidine and histamine derivative Nω-acetylhistamine as analytes. The concentration range of the analytes applied was the same as for histamine. From the analytes tested putrescine was the only analyte that inhibited the binding of Ab1 to the probes, and the cross-reactivity was determined to be 4.2%. The cross-reactivity behavior is in concordance with the results from Adányi et al. [32], who applied the same Ab1 in OWLS-based immunosensor and reported that the highest and only significant interference was for putrescine (13.6%).

## 4. Discussion

We tested six commercial antibodies, from which only two bound the histamine free in the solution. One of these antibodies (Ab2) exhibited high affinity towards the carried protein itself and the binding of the probes by Ab2 was only weakly inhibited upon addition of free histamine. In fact, Ab2 was recommended for use in IHC and it has been applied in several successful studies in the field [43–45]. However, with Ab1, we also obtained low sensitivity compared to the requirements for analyzing food spoilage in real samples, typically ranging from 2.5 to 500 ppm histamine for fish products. This was not surprising though as the Ab1 was also developed for IHC applications where the antibody is supposed to bind the histamine present in the fixed tissue. Nevertheless, the same anti-histamine antibody Ab1 from Sigma Aldrich (H7403) was used by Adányi et al. [32] and Yang et al. [34] (personal communication) with higher sensitivities, but in completely different set-ups: direct and competitive assay using optical waveguide lightmode spectroscopy detection [32] and competitive assay with electrochemical detection on modified graphene [34].

The chemical structure of the immobilized competitor hapten and the hapten used for immunization has a crucial role in the assay development. In heterologous assays, the immunizing and competitor haptens differ in their structure, whereas, in the homologous assays, the haptens are the same; the assay sensitivity of such heterologous assays is reported to be better than in homologous assay set-ups [18,46–48]. KLH-EDC-his was used as probe as well as to generate Ab1; thus, expectedly, the affinity of Ab1 was high towards the protein-EDC-his conjugates. However, controversial to the hypothesis based on heterology no significant improvement in the assay sensitivity was obtained by using a competing hapten-conjugate different from the immunization conjugate. Nevertheless, the heterology concept for small haptens with extremely simple structure such as histamine is challenging to establish as shown by Luo et al. [18], who synthesized several conjugates with derivatized histamine in order to obtain an antibody with specific histamine affinity. As none of the produced antisera recognized free histamine and the final assay setup required a histamine derivatization step, the assay sensitivity improvement could only be shown with one heterologous competitor hapten. Moreover, polyclonal antibodies usually exhibit affinity towards several epitopes. It has been claimed that only 0.5–5% of the antibodies in a polyclonal reagent bind to their intended target [25].

Poor reproducibility of scientific studies has recently raised discussion especially about the quality of the commercial antibodies. Several authors have addressed the issue and claimed for better characterization and profound information on the specificity and cross-reactivity of the antibodies [19–28]. The antibodies in the market are often insufficiently characterized and furthermore they might possess batch-to-batch variation, usually in much larger extent with polyclonal antibodies compared to monoclonal. The vendors are not still required to verify the quality of every new batch [49]. In an alerting notion Weller [20] hypothesized that high quality and well-characterized antibodies developed by academic groups are often sold to test kit manufacturers. Consequently, the antibodies available in the free market might be the ones with doubtful quality and insufficient information about their performance.

Several databases exist to ease the antibody selection and to provide more information with references to published literature, and authors are encouraged to use the references to the databases. For instance, Antibody Registry [40] is an antibody reagent portal listing commercial antibodies carrying unique identification code, which we employed in this study. However, in general most common applications listed in antibody databases are western blot assays, IHC and ICC [50]. All the antibodies used in the study except one were listed in the database [40], but only the Ab2 was linked to references in literature of which all considered IHC or ICC applications. At present, some databases such as antibodypedia.com or pabmabs.com had no entries for histamine specific antibodies. The common databases should prevent the overlapping supply between the different vendors; nonetheless, the same antibody can be sold by different providers just with different labels and product number and users might accidentally buy the same reagent from different distributors [19,28]. Thus, each antibody should be adequately identified and linked to a code that should be kept unaltered by users and vendors. The traceability of the antibodies remains a major challenge in regard identifying antibodies used in published literature but also consolidating and updating characterization data to a correct product. We also encountered a traceability issue with the anti-histamine antibodies with respect to literature as well as vendors. Several publications failed to provide the catalogue number or identification of the antibody used in the study (however, all the authors provided the information when inquired). We ordered two polyclonal antibodies with similar specifications from different vendors: Ab4 from Biozol and polyclonal anti-histamine antibody from AbD Serotec (nowadays part of Bio-Rad). Upon further investigation, we figured out that the antibodies were the same and originally from the same manufacturer. The antibody from AbD Serotec/Bio-Rad was withdrawn due to the quality issues, and later on the item was discontinued from Biozol as well. Furthermore, Ab3 is also discontinued and not available for purchasing anymore.

Even though there is a high demand for better antibody characterization, it is not economically attractive to perform the characterization for all the existing items [29], and furthermore to characterize the batch-to-batch variability. Moreover, the characterization only describes the performance of the antibody in the tested application method and thus it is not guaranteed to have the same binding efficiency in other set-ups. The intended application for each product is usually stated by the manufacturer but the detailed assay setup or references are rarely provided. For instance, in the case of Ab6 the supplier did not promise the antibody to bind the free histamine but recommended the user to test the specificity on their intended assay setup (personal communication).

In conclusion, there is a need for improved characterization of commercial antibodies and availability of detailed specifications to the users. Nevertheless, several antibody producers and vendors are aware of the antibody quality problem, but characterization of all the catalogued antibodies would not be economically worthy. We suggest that the research groups should provide the detailed information of the antibody used, such as the catalogue number and the batch or lot as well as information about the immunogen preparation, for reproducibility of the scientific data but also for the manufacturers to know which one of their products was used and what was its performance in a given application. Furthermore, vendors should further encourage the scientific community to provide user evaluations. Many suppliers already offer small trial amounts for certain antibodies for cheaper prices. However, the service of sample size antibodies could be extended to also less common antibodies, and, in cases where several antibodies are catalogued for the same analyte, to enable customers to find the most suitable antibody for their application. The high quality of the commercial antibodies is in the interests of both the vendors and the researchers to provide and publish good quality and reproducible data.

**Supplementary Materials:** The following are available online at www.mdpi.com/2227-9040/5/4/33/s1, Figure S1: Fluorescence response upon addition of increasing amounts of anti-histamine antibodies onto the BSA-EDC-his probe, Figure S2: Fluorescence signals of the zero analyte concentration on protein-EDC-his and pure protein.

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**Author Contributions:** Claudia Preininger conceived and designed the experiments; Leena Mattsson performed the experiments and analyzed the data; Sara Doppler contributed in reagents, materials and analysis tools; and Leena Mattsson and Claudia Preininger wrote the paper.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Silla Santos, M.H. Biogenic amines: Their importance in foods. *Int. J. Food Microbiol.* **1996**, *29*, 213–231. [CrossRef]
2. Food and Drug Administration. Fish and Fishery Products Hazards and Controls Guidance. Fourth edition—April 2011. Available online: http://www.fda.gov/FoodGuidances (accessed on 5 December 2017).
3. Morel, A.; Darmon, M.; Delaage, M. Recognition of imidazole and histamine derivatives by monoclonal antibodies. *Mol. Immunol.* **1990**, *27*, 995–1000. [CrossRef]
4. Morel, A.M.; Delaage, M.A. Immunoanalysis of histamine through a novel chemical derivatization. *J. Allergy Clin. Immunol.* **1988**, *82*, 646–654. [CrossRef]
5. Guesdon, J.L.; Chevrier, D.; Mazie, J.C.; David, B.; Avrameas, S. Monoclonal anti-histamine antibody. Preparation, characterization and application to enzyme immunoassay of histamine. *J. Immunol. Methods* **1986**, *87*, 69–78. [CrossRef]
6. Chevrier, D.; Guesdon, J.L.; Mazie, J.C.; Avrameas, S. Enzyme immunoassay for the measurement of histamine. *J. Immunol. Methods* **1986**, *94*, 119–125. [CrossRef]
7. Rauch, P.; Rychetsky, P.; Hochel, I.; Bilek, R.; Guesdon, J. Enzyme immunoassay of histamine in foods. *Food Agric. Immunol.* **1992**, *4*, 67–72. [CrossRef]

8. Serrar, D.; Brebant, R.; Bruneau, S.; Denoyel, G.A. The development of a monoclonal antibody-based ELISA for the determination of histamine in food: Application to fishery products and comparison with the HPLC assay. *Food Chem.* **1995**, *54*, 85–91. [CrossRef]
9. Haydik, I.B. Histamine determination by radioimmunoassay (RIA) [Abstract]. *J. Allergy Clin. Immunol.* **1983**, *71*, 152. [CrossRef]
10. Buckler, R.T.; Dailey, F.A.; Ficalora, J.A.; Gavin, J.J.; Plunkett, G.A. Histamine derivatives, immunogen conjugates and antibodies raised thereto. Patent US5112738, 12 May 1992.
11. Panula, P.; Yang, H.; Costa, E. Histamine-containing neurons in the rat hypothalamus. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 2572–2576. [CrossRef] [PubMed]
12. Dohlsten, M.; Hallberg, T.; Håkanson, R. Production and characterization of rabbit antibodies against histamine. *Mol. Immunol.* **1986**, *23*, 799–806. [CrossRef]
13. Mita, H.; Yasueda, H.; Shida, T.; Baba, S. An attempt to produce an antibody of histamine and histamine derivatives. *Agents Actions* **1984**, *14*, 574–579. [CrossRef] [PubMed]
14. Wilcox, B.J.; Seybold, V.S. Localization of neuronal histamine in rat brain. *Neurosci. Lett.* **1982**, *29*, 105–110. [CrossRef]
15. Johansson, O.; Virtanen, M.; Hilliges, M.; Yang, Q. Histamine immunohistochemistry: A new and highly sensitive method for studying cutaneous mast cells. *Histochem. J.* **1992**, *24*, 283–287. [CrossRef] [PubMed]
16. Schneider, E.; Usleber, E.; Märtlbauer, E. Production and characterization of antibodies against histamine. In *Immunoassays for Residue Analysis*; ACS Publications: Washington, DC, USA, 1996; pp. 413–420, ISBN 9780841233799.
17. Aygün, O.; Schneider, E.; Scheuer, R.; Usleber, E.; Gareis, M.; Märtlbauer, E. Comparison of ELISA and HPLC for the determination of histamine in cheese. *J. Agric. Food Chem.* **1999**, *47*, 1961–1964. [CrossRef] [PubMed]
18. Luo, L.; Xu, Z.-L.; Yang, J.-Y.; Xiao, Z.-L.; Li, Y.-J.; Beier, R.C.; Sun, Y.-M.; Lei, H.-T.; Wang, H.; Shen, Y.-D. Synthesis of novel haptens and development of an enzyme-linked immunosorbent assay for quantification of histamine in foods. *J. Agric. Food Chem.* **2014**, *62*, 12299–12308. [CrossRef] [PubMed]
19. Baker, M. Blame It on the antibodies. *Nature* **2015**, *521*, 274–276. [CrossRef] [PubMed]
20. Weller, M.G. Quality Issues of Research Antibodies. *Anal. Chem. Insights* **2016**, *11*, 21–27. [CrossRef] [PubMed]
21. Baker, M. Antibody anarchy: A call to order. *Nature* **2015**, *527*, 545–551. [CrossRef] [PubMed]
22. Beermann, S.; Seifert, R.; Neumann, D. Commercially available antibodies against human and murine histamine H4-receptor lack specificity. *Naunyn. Schmiedebergs. Arch. Pharmacol.* **2012**, *385*, 125–135. [CrossRef] [PubMed]
23. Saper, C.B. An open letter to our readers on the use of antibodies. *J. Comp. Neurol.* **2005**, *493*, 477–478. [CrossRef] [PubMed]
24. Pradidarcheep, W.; Labruyère, W.T.; Dabhoiwala, N.F.; Lamers, W.H. Lack of specificity of commercially available antisera: Better specifications needed. *J. Histochem. Cytochem.* **2008**, *56*, 1099–1111. [CrossRef] [PubMed]
25. Bradbury, A.; Plückthun, A. Reproducibility: Standardize antibodies used in research. *Nature* **2015**, *518*, 27–29. [CrossRef] [PubMed]
26. O'Kennedy, R.; Fitzgerald, S.; Murphy, C. Don't blame it all on antibodies—The need for exhaustive characterisation, appropriate handling, and addressing the issues that affect specificity. *TrAC Trends Anal. Chem.* **2017**, *89*, 53–59. [CrossRef]
27. Bordeaux, J.; Welsh, A.; Agarwal, S.; Killiam, E.; Baquero, M.; Hanna, J.; Anagnostou, V.; Rimm, D. Antibody validation. *Biotechniques* **2010**, *48*, 197–209. [CrossRef] [PubMed]
28. Roncador, G.; Engel, P.; Maestre, L.; Anderson, A.P.; Cordell, J.L.; Cragg, M.S.; Šerbec, V.Č.; Jones, M.; Lisnic, V.J.; Kremer, L.; Li, D.; Koch-Nolte, F.; Pascual, N.; Rodríguez-Barbosa, J.-I.; Torensma, R.; Turley, H.; Pulford, K.; Banham, A.H. The European antibody network's practical guide to finding and validating suitable antibodies for research. *MAbs* **2016**, *8*, 27–36. [CrossRef] [PubMed]
29. Voskuil, J.L.A. The challenges with the validation of research antibodies. *F1000Research* **2017**, *6*, 161. [CrossRef] [PubMed]
30. Vasilevsky, N.A.; Brush, M.H.; Paddock, H.; Ponting, L.; Tripathy, S.J.; LaRocca, G.M.; Haendel, M.A. On the reproducibility of science: Unique identification of research resources in the biomedical literature. *PeerJ.* **2013**, *1*, e148. [CrossRef] [PubMed]

31. Li, Y.; Kobayashi, M.; Furui, K.; Soh, N.; Nakano, K.; Imato, T. Surface plasmon resonance immunosensor for histamine based on an indirect competitive immunoreaction. *Anal. Chim. Acta* **2006**, *576*, 77–83. [CrossRef] [PubMed]
32. Adányi, N.; Székács, I.; Szendro, I.; Székács, A. Determination of histamine content in vegetable juices by using direct and competitive immunosensors. *Food Agric. Immunol.* **2014**, *25*, 37–41. [CrossRef]
33. Lim, T.K.; Ohta, H.; Matsunaga, T. Microfabricated on-chip-type electrochemical flow immunoassay system for the detection of histamine released in whole blood samples. *Anal. Chem.* **2003**, *75*, 3316–3321. [CrossRef] [PubMed]
34. Yang, M.; Zhang, J.; Chen, X. Competitive electrochemical immunosensor for the detection of histamine based on horseradish peroxidase initiated deposition of insulating film. *J. Electroanal. Chem.* **2015**, *736*, 88–92. [CrossRef]
35. Köse, S.; Kaklikkaya, N.; Koral, S.; Tufan, B.; Buruk, K.C.; Aydin, F. Commercial test kits and the determination of histamine in traditional (ethnic) fish products-evaluation against an EU accepted HPLC method. *Food Chem.* **2011**, *125*, 1490–1497. [CrossRef]
36. Rogers, P.L.; Staruszkiewicz, W.F. Histamine Test Kit Comparison. *J. Aquat. Food Prod. Technol.* **2000**, *9*, 5–17. [CrossRef]
37. Gogalic, S.; Sauer, U.; Doppler, S.; Heinzel, A.; Perco, P.; Lukas, A.; Simpson, G.; Pandha, H.; Horvath, A.; Preininger, C. Validation of a protein panel for the non-invasive detection of recurrent non-muscle invasive bladder cancer. *Biomarkers* **2017**, *19*, 1–8. [CrossRef] [PubMed]
38. Buchegger, P.; Preininger, C. Four Assay Designs and On-Chip Calibration: Gadgets for a Sepsis Protein Array. *Anal. Chem.* **2014**, *86*, 3174–3180. [CrossRef] [PubMed]
39. Poller, A.M.; Crooks, S.; Preininger, C. Influence of different surface chemistries on the ultrasensitive on-chip detection of enrofloxacin in milk. *Sens. Actuators B Chem.* **2015**, *209*, 1077–1083. [CrossRef]
40. The Antibody Registry. Available online: http://antibodyregistry.org/ (accessed on 14 October 2017).
41. Strasser, A.; Dietrich, R.; Usleber, E.; Märtlbauer, E. Immunochemical rapid test for multiresidue analysis of antimicrobial drugs in milk using monoclonal antibodies and hapten-glucose oxidase conjugates. *Anal. Chim. Acta* **2003**, *495*, 11–19. [CrossRef]
42. Fujiwara, K.; Kitagawa, T.; Inoue, Y.; Alonso, G. Monoclonal antibodies against glutaraldehyde-conjugated histamine: Application to immunocytochemistry. *Histochem. Cell Biol.* **1997**, *107*, 39–45. [CrossRef] [PubMed]
43. Romero-Calderón, R.; Uhlenbrock, G.; Borycz, J.; Simon, A.F.; Grygoruk, A.; Yee, S.K.; Shyer, A.; Ackerson, L.C.; Maidment, N.T.; Meinertzhagen, I.A.; et al. A glial variant of the vesicular monoamine transporter is required to store histamine in the Drosophila visual system. *PLoS Genet.* **2008**, *4*. [CrossRef]
44. McCoole, M.D.; Baer, K.N.; Christie, A.E. Histaminergic signaling in the central nervous system of Daphnia and a role for it in the control of phototactic behavior. *J. Exp. Biol.* **2011**, *214*, 1773–1782. [CrossRef] [PubMed]
45. Borycz, J.; Borycz, J.A.; Loubani, M.; Meinertzhagen, I.A. tan and ebony genes regulate a novel pathway for transmitter metabolism at fly photoreceptor terminals. *J. Neurosci.* **2002**, *22*, 10549–10557. [PubMed]
46. Wortberg, M.; Goodrow, M.H.; Gee, S.J.; Hammock, B.D. Immunoassay for Simazine and Atrazine with Low Cross-Reactivity for Propazine. *J. Agric. Food Chem.* **1996**, *44*, 2210–2219. [CrossRef]
47. Holthues, H.; Pfeifer-Fukumura, U.; Sound, I.; Baumann, W. Evaluation of the concept of heterology in a monoclonal antibody-based ELISA utilizing direct hapten linkage to polystyrene microtiter plates. *J. Immunol. Methods* **2005**, *304*, 68–77. [CrossRef] [PubMed]
48. Goodrow, M.H.; Hammock, B.D.; Harrison, R.O. Hapten synthesis, antibody development, and competitive inhibition enzyme immunoassay for s-triazine herbicides. *J. Agric. Food Chem.* **1990**, *38*, 990–996. [CrossRef]
49. Rizner, T.L.; Sasano, H.; Choi, M.H.; Odermatt, A.; Adamski, J. Recommendations for description and validation of antibodies for research use. *J. Steroid Biochem. Mol. Biol.* **2016**, *156*, 40–42. [CrossRef] [PubMed]
50. Uhlen, M.; Bandrowski, A.; Carr, S.; Edwards, A.; Ellenberg, J.; Lundberg, E.; Rimm, D.L.; Rodriguez, H.; Hiltke, T.; Snyder, M.; Yamamoto, T. A proposal for validation of antibodies. *Nat. Methods* **2016**, *13*. [CrossRef] [PubMed]